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Effect of the Ecdysone Agonists, RH-2485 and Tebufenozide, on the Southwestern Corn Borer, *Diatraea grandiosella*

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Abstract: The effect of the ecdysone agonists RH-2485 (proposed name methoxyfenozide) and tebufenozide (RH-5992), was examined on eggs and larvae of the southwestern corn borer, *Diatraea grandiosella* Dyar. Both compounds exhibited a concentration-dependent ovicidal activity. More than 95% of eggs died when egg masses were dipped in solutions of 100 or 200 mg liter^{-1} of either compound in acetone + distilled water (1 + 1 by volume). Although some eggs treated with 1 or 10 mg liter^{-1} of the compounds hatched, the survival rate was low. Newly hatched larvae were fed for seven days on an artificial diet containing RH-2485 or tebufenozide. The LC_{50} values were 0·049 mg kg^{-1} for RH-2485 and 0·185 mg kg^{-1} for tebufenozide, showing that RH-2485 was about four times more active than was tebufenozide. Although increasing the time of exposure to either compound decreased the LC_{50} value significantly, the relative potency of RH-2485 versus tebufenozide was not changed. Newly ecdysed 4th-instar larvae fed with diets containing 0·125, 0·25 or 0·5 mg kg^{-1} RH-2485 or tebufenozide ceased feeding approximately 8 h after exposure, indicating that larvae had prematurely entered a molting cycle. Larvae treated with RH-2485 ecdysed earlier and died more quickly than those treated with tebufenozide. Ingestion of sublethal concentrations of RH-2485 (0·005 and 0·01 mg kg^{-1}) or tebufenozide (0·03 and 0·06 mg kg^{-1}) retarded larval growth, and decreased pupal weight and adult emergence. Increasing exposure time to tebufenozide tended to increase the larval mortality, significantly retarded larval growth, and decreased the mean weights of male and female pupae and adult emergence. RH-2485 (0·125 and 0·25 mg kg^{-1}) and tebufenozide (0·25 and 0·5 mg kg^{-1}) were lethal to newly hatched larvae, even after diets containing these compounds were held for 20 days at 30°C under long days (16 h light : 8 h dark). Our results suggest that field trials to assess the potential of RH-2485 and tebufenozide to control *D. grandiosella* are warranted. © 1998 SCI

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Key words: *Diatraea grandiosella;* ecdysone agonists; RH-2485; methoxyfenozide; RH-5992; tebufenozide

1 INTRODUCTION

The southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Pyralidae), is present in Mexico and the southern United States and can cause serious yield losses to maize, especially when the crop is irrigated. Stalk feeding of the late instars causes substantial injury to the maize plant, and girdling causes lodging of the stalk so that ears cannot be harvested by machine.[1] Stalk girdling by *D. grandiosella* was estimated at 80–85% in Alabama (1976),[2] and yield loss of 29 bushels acre^{-1} was reported in Kentucky in 1976.[3] Control of *D. grandiosella* has relied on cultural practices and conventional insecticides that have a broad

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spectrum of activity. A need exists to develop effective insect growth regulators (IGRs) against *D. grandiosella* that are compatible with integrated pest management practices.

RH-5849 (*N-tert*-butyl-N′-benzoyl-benzohydrazide) is the prototype of ecdysone agonists that have potential for the control of pest Lepidoptera,[4] and have low toxicity to non-target insects.[5,6] Tebufenozide (RH-5992) and RH-2485 (proposed name methoxyfenozide) are other ecdysone agonists that have been reported to be more lethal against lepidopterous larvae than is RH-5849. Tebufenozide was 60–75 times more lethal than was RH-5849 to the nutgrass armyworm, *Spodoptera exempta* (Walker), the beet armyworm, *S. exigua* (Hübner), and the cotton armyworm, *S. littoralis* (Boisduval).[7,8] RH-2485 was 3–7 times more lethal than was tebufenozide to *S. littoralis*,[9] the pandemis leafroller, *Pandemis pyrusana* Kearfott, the obliquebanded leafroller, *Choristoneura rosaceana* (Harris),[10] and the European corn borer, *Ostrinia nubilalis* (Hübner).[11] These compounds bind to ecdysteroid receptors and provide larvae with a premature signal to start synthesizing cuticle before they are competent to molt.[4,12] The present study examined the effects of RH-2485 and tebufenozide on eggs and larvae of *D. grandiosella.*

2 MATERIALS AND METHODS

2.1 Insects

A colony of *D. grandiosella* was maintained on a BioServ artificial diet in 1-oz clear plastic cups using established laboratory procedures.[13] Larvae were reared under long days (16 : 8 h light : dark) at 30°C. Pupae, adults and eggs were held at 13 : 11 h light : dark at 25°C.

2.2 Chemicals

Technical RH-2485 (*N-tert*-butyl-*N′*(3-methoxy-2-methylbenzoyl)-3,5-dimethylbenzohydrazide, ~ 94%) and tebufenozide (*N-tert*-butyl-N′-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide, 95%) were obtained from Rohm and Haas Company (Spring House, PA). Analytical grade acetone was used.

2.3 Ovicidal activity

Four concentrations, 200, 100, 10 and 1 mg liter^{-1} RH-2485 or tebufenozide were prepared in acetone + distilled water (1 + 1 by volume). Solvent and untreated controls were included in the assay. Wax paper containing egg masses (one to three days old) was cut and dipped in the solution for 30 s, air dried and transferred into plastic cups. Moist cotton containing 0·6 ml water was placed in the cups. At least 50 eggs from several egg masses were used for each treatment, and each treatment was replicated three times. The number of newly hatched larvae from each treatment was recorded daily. If sufficient larvae were available, 20 from each treatment were selected randomly from larvae that hatched on the first day of hatching for observations of subsequent larval survival. Larval mortality was determined at 10 days after larvae had been fed with fresh diet. Percentage egg and larval mortality data were transformed using arcsine $\sqrt{percentage}$ before being submitted to analysis of variance (ANOVA).[14] ANOVA was carried out using a completely randomized design (CRD), and least significant difference (LSD) with $\alpha = 0{\cdot}05$ was applied for means comparison only when the F-test ($\alpha = 0{\cdot}05$) in the ANOVA was significant (Fisher protected LSD). Statistical analyses were carried out using MSTAT.[15]

2.4 Larval toxicity

The LC_{50} values of RH-2485 and tebufenozide were determined for newly hatched larvae (<1 day old). Serial solutions of the compounds were prepared in acetone, and a test solution (2 ml) was incorporated into 700 g larval diet during its preparation. Control diet was treated with 2 ml acetone. Acetone was evaporated by placing the BioServ Dry Mix containing the test solution under a hood for 10–15 min. On the basis of a preliminary assay, at least four concentrations of the compounds that caused mortality ranging from 5–97% were used to determine their LC_{50} values. Forty-five to 50 newly hatched larvae with three replicates were used for each concentration. Larvae were transferred individually into the plastic cups containing a cube (approximately 12 g) of treated or control diet. Larvae were fed continuously on treated or control diets, and mortality was observed 7, 10, 14 and 18 days after treatment. Larvae were considered dead if they did not move when they were probed using a paint-brush. Data were submitted to probit analysis using MSTAT.[15]

2.5 Effect on larval growth and molting

Newly ecdysed 4th-instar larvae (<1 day old) were fed with diets containing three concentrations of RH-2485 or tebufenozide (0·5, 0·25 and 0·125 mg kg^{-1}). Control diet was treated with acetone. Diets were prepared as described earlier. Eleven larvae, each as a replicate, were used per treatment for RH-2485, and 10 larvae per treatment for tebufenozide. They were weighed individually before being released on to treated or control diets. Ecdysis and weight gain for each surviving larva were observed at 8-h intervals during the first two days and at 12- or 24-h intervals for the following days until

120 h after treatment. Observation on the larval mortality was continued until 144 h after treatment. CRD was used for ANOVA, and Fisher protected LSD for equal or unequal replicates with $\alpha = 0{\cdot}05$ was applied to compare means of larval weight. Statistical analyses were carried out using MSTAT.[15]

2.6 Chronic effects

The effect of RH-2485 applied at sublethal concentrations (0·005 and 0·01 mg kg^{-1}, the expected LC_5 and LC_{20} at 18 days after treatment, respectively) on larval mortality and growth, pupal weight and adult emergence was determined. Control diet was treated with acetone only. Newly hatched larvae were transferred individually into separate plastic cups containing a cube of treated or control diet. Thirty-four to 41 larvae were used for each treatment, and each treatment was replicated three times. Larvae were continuously fed with treated or control diet. On day 28, surviving larvae from all treatments were transferred on to fresh control diet.

Two concentrations of tebufenozide (0·06 and 0·03 mg kg^{-1}) were selected to give approximately 45% and 15% larval mortality, respectively. Control diet was treated with acetone. Twenty-five to 50 newly hatched larvae were placed individually in plastic cups containing a cube of treated or control diet for eight and 28 days. Each treatment was replicated three times. Larvae from treatment of eight-day exposure were transferred on to fresh control diet after they had fed on treated or control diet for eight days. On day 28, surviving larvae from all treatments were then transferred on to fresh control diet.

RH-2485 and tebufenozide experiments were terminated at 56 days after treatment. At this time, >90% of surviving larvae in all treatments had ecdysed into pupae. Total larval mortality was computed by dividing the number of dead larvae by number of larvae used in each treatment. The number of larvae was determined by subtracting from the initial number of larvae the number of surviving larvae (two or fewer) found in each treatment at the end of the experiment. Male and female pupae were collected daily and weighted separately. Pupal eclosion was observed until the third week after the last collection of pupae. Adult emergence was determined by dividing the number of adults by the number of pupae and expressed as percentage. Percentage data were transformed before being submitted into ANOVA.[14] Percentage larval mortality data due to RH-2485 were transformed using square root transformation because the mortality was <30%, and adult emergence (%) was transformed using arcsine $\sqrt{percentage}$. Percentage larval mortality data due to tebufenozide and adult emergence (%) were transformed using arcsine $\sqrt{percentage}$. ANOVA was carried out using a CRD for RH-2485 and a 3×2 factorial arrangement for tebufenozide. Fisher protected LSD with $\alpha = 0{\cdot}05$ was used for means separation. Statistical analyses were carried out using MSTAT,[15] and original data are presented.

2.7 Stability of the ecdysone agonists in the diet

The effect of RH-2485 (0·25 and 0·125 mg kg^{-1}) and tebufenozide (0·5 and 0·25 mg kg^{-1}) on newly hatched larvae was examined after the diets were held for 0, 5, 10, 15 and 20 days at 30°C, 16 : 18 h light : dark. Control diet was treated with 2 ml acetone. Ten larvae were transferred individually onto treated or control diets held for 0, 5, 10, 15 or 20 days, and each treatment was replicated three times. Mortality was recorded daily, and observation was terminated seven days after treatment because all larvae had died.

3 RESULTS

3.1 Ovicidal effects

RH-2485 and tebufenozide exhibited ovicidal activity in a concentration-dependent fashion (Table 1). Of the eggs treated with 100 and 200 mg $liter^{-1}$ RH-2485 or tebufenozide 99–100% died without showing embryonic development. The larvae that hatched from eggs treated with 100 and 10 mg $liter^{-1}$ RH-2485 or 200 mg $liter^{-1}$ tebufenozide died the day after they were transferred on to fresh larval diet. Application of 10 and 1 mg $liter^{-1}$ of these compounds to eggs caused significant egg mortality and decreased the survival rate of larvae.

3.2 Larval toxicity

RH-2485 was about four times more lethal to newly hatched larvae than was tebufenozide (Table 2), and its relative toxicity did not change with increasing time of observation. Larvae usually died during the 1st molting cycle. Some treated larvae continued to feed at a lower rate than did the control larvae, which eventually caused additional deaths. The slopes increased with increasing time of observations. This progressive mortality resulted in a significant decrease in the LC_{50} values.

3.3 Effect on growth and molting

There were no significant differences between the weight gains of newly ecdysed 4th-instar larvae fed for 8 h with diets containing 0·125, 0·25 or 0·5 mg kg^{-1} RH-2485 (Fig. 1) or tebufenozide (Fig. 2) and those fed with the control diet. However, observation at 16 h after feeding

TABLE 1
Effect of RH-2485 and Tebufenozide on One- to Three-Day-Old Eggs of *Diatraea grandiosella*

Ecdysone agonist	*Concentration (mg liter^{-1})*	*Eggs (no.)*	*Egg mortality*[a] (%) (±*S.D.*)	*Larvae (no.)*	*Larval mortality*[a, b] (%) (±*S.D.*)
RH-2485	200	282	100·0 (±0·0)a		
	100	253	99·6 (±0·6)a		
	10	260	96·3 (±3·3)a		
	1	252	48·5 (±18·5)b	60	31·7 (±5·8)a
	Solvent control	222	18·9 (±2·2)c	60	10·0 (±5·0)b
	Untreated control	249	12·8 (±2·7)c	60	6·7 (±2·9)b
Tebufenozide	200	323	99·0 (±1·7)a		
	100	307	100·0 (±0·0)a		
	10	332	70·7 (±10·1)b	60	15·0 (±5·0)a
	1	313	56·1 (±13·4)b	60	5·0 (±5·0)b
	Solvent control	266	15·2 (±4·0)c	56	0b
	Untreated control	220	15·2 (±2·0)c		

[a] Means within columns for each ecdysone agonist followed by the same letter are not significantly different, Fisher protected LSD; $P > 0{\cdot}05$.
[b] Mortality was determined after larvae had fed for 10 days on control diet.

showed that the mean weight of larvae treated with RH-2485 or tebufenozide was significantly lower than that of control larvae ($P < 0{\cdot}05$). This finding suggests that larvae treated with RH-2485 or tebufenozide stopped feeding approximately 8 h after exposure and had prematurely entered a molting cycle. Observations taken between 24 and 120 h after feeding showed that the mean weight of treated larvae continued to be significantly lower than that of control larvae ($P < 0{\cdot}05$). Even though the mean weight of larvae treated with 0·5 mg kg^{-1} tebufenozide was significantly lower than that of those treated with 0·125 or 0·25 mg kg^{-1} tebufenozide at 60, 84 and 96 h after feeding ($P < 0{\cdot}05$), no significant difference was detected at 120 h after feeding. Surviving larvae from 0·25 and 0·5 mg kg^{-1} RH-2485 or tebufenozide treatments did not gain additional weight until the last observation. On the other hand, surviving larvae from the 0·125 mg kg^{-1} RH-2485 or tebufenozide gained additional weight.

RH-2485 and tebufenozide applied to newly ecdysed 4th-instar larvae induced a premature and lethal molting cycle (Figs 3 and 4). Larvae treated with RH-2485 ecdysed to the 5th instars earlier than those treated with tebufenozide (Fig. 3). The first ecdysis occurred at 24 h after feeding in all RH-2485 treatments, whereas the first ecdysis was observed 32 h after feeding in the 0·5 mg kg^{-1} tebufenozide treatment, and 40 h after feeding in the 0·25 and 0·125 mg kg^{-1} tebufenozide treatments. Increasing the concentration of RH-2485 or tebufenozide shortened the time to ecdyse. All larvae ecdysed to the 5th instar by 40 h after feeding

TABLE 2
Relative Toxicities of RH-2485 and Tebufenozide to Newly Hatched Larvae of *Diatraea grandiosella*

Ecdysone agonist[a]	*Larvae (no.)*	*Time (days)*	*Control mortality (%)*	*Slope (±SE)*	*LC_{50} (95% CL)*[b] *(mg kg^{-1})*	χ^2	*Relative toxicity*[c]
RH-2485	687	7	0	2·08 (±0·21)	0·049a (0·042–0·058)	2·39	3·8
		10	0·7	2·57 (±0·21)	0·024b (0·023–0·028)	2·28	4·4
		14	0·7	2·79 (±0·22)	0·021bc (0·019–0·023)	0·20	3·5
		18	0·7	2·94 ±0·23)	0·018c (0·016–0·020)	0·25	3·6
Tebufenozide	748	7	0	2·26 (±0·20)	0·185a (0·160–0·225)	10·61	
		10	0	2·73 (±0·20)	0·106b (0·096–0·117)	0·25	
		14	0	3·12 (±0·21)	0·074cd (0·068–0·081)	0·20	
		18	0	3·21 (±0·22)	0·064d (0·059–0·070)	0·33	

[a] The ecdysone agonist was incorporated into the artificial diet.
[b] LC_{50} values followed by the same letter within the ecdysone agonist are not significantly different based on non-overlapping 95% CL.
[c] The relative toxicity of RH-2485 compared with that of tebufenozide (LC_{50} of tebufenozide divided by LC_{50} of RH-2485).

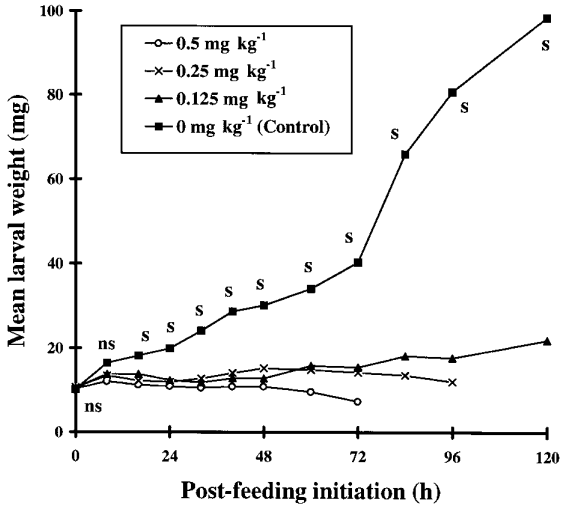

Fig. 1. Effect of RH-2485 on weight gain of 4th-instar (<1 day old) larvae of *Diatraea grandiosella* maintained at 30°C, 16 : 8 h light : dark. $n = 11$ larvae per treatment. ns = the mean weight of control larvae was not significantly different from that of larvae treated with 0·125, 0·25 or 0·5 mg kg^{-1} RH-2485. s = the mean weight of control larvae was significantly higher than that of larvae treated with 0·125, 0·25 or 0·5 mg kg^{-1} RH-2485, Fisher protected LSD; $P < 0{\cdot}05$.

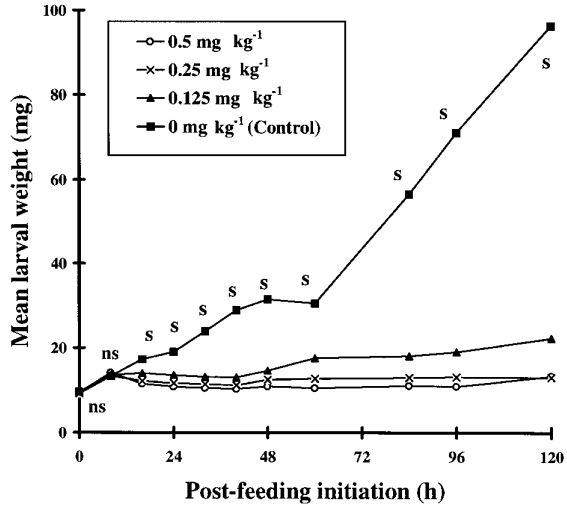

Fig. 2. Effect of tebufenozide on weight gain of 4th-instar (< 1 day old) larvae of *Diatraea grandiosella* maintained at 30°C, 16 : 8 h light : dark. $n = 10$ larvae per treatment. ns = the mean weight of control larvae was not significantly different from that of larvae treated with 0·125, 0·25 or 0·5 mg kg^{-1} tebufenozide. s = the mean weight of control larvae was significantly higher than that of larvae with 0·125, 0·25 or 0·5 mg kg^{-1} tebufenozide, Fisher protected LSD; $P < 0{\cdot}05$.

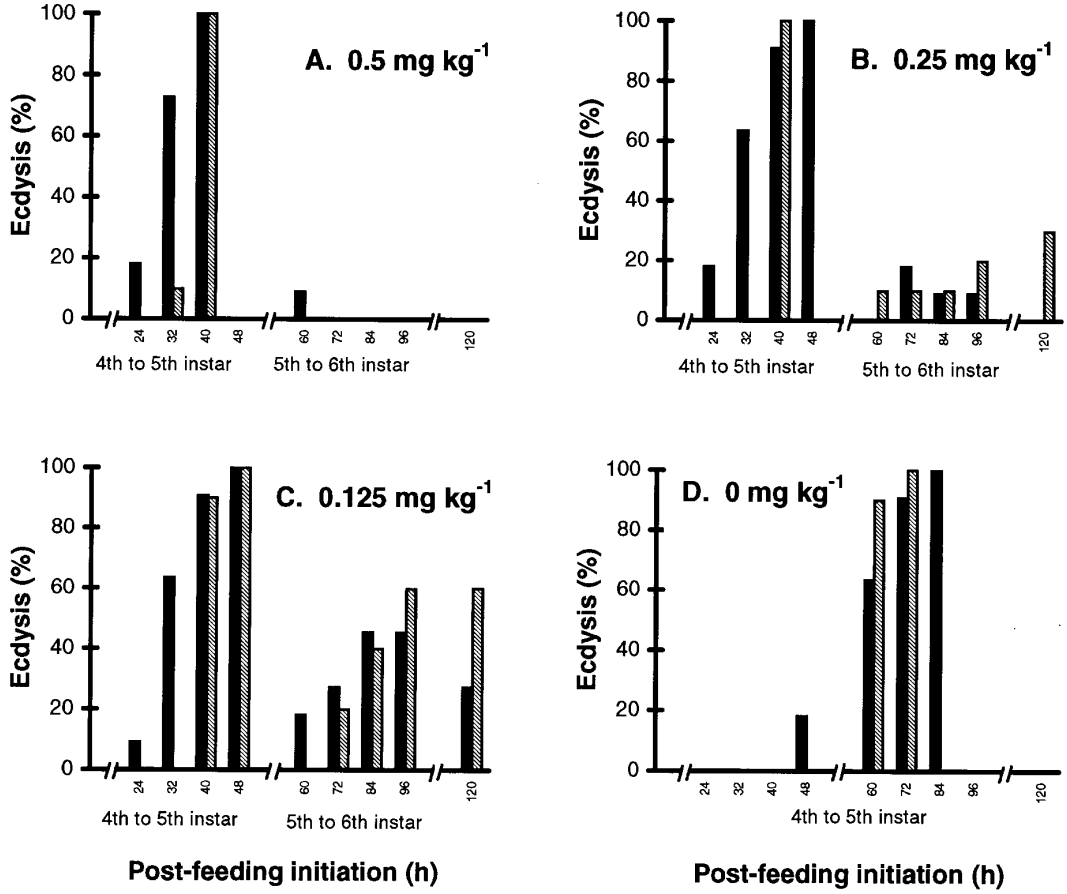

Fig. 3. Effect of (■) RH-2485 and (▨) tebufenozide administered in artificial diet on ecdysis of newly ecdysed 4th-instar (<1 day old) larvae of *Diatraea grandiosella* maintained at 30°C, 16 : 8 h light : dark. Observation was carried out with 8 h interval in the first two days and 12 h interval in the second two days after exposure. $n = 11$ larvae per treatment for RH-2485, and $n = 10$ larvae per treatment for tebufenozide.

with diets containing 0·5 mg kg^{-1} tebufenozide or RH-2485. In the 0·125 mg kg^{-1} treatment, all larvae ecdysed to the 5th instar by 48 h after feeding was initiated. Decreasing the concentration of RH-2485 or tebufenozide increased the number of larvae that underwent an additional ecdysis into the 6th instar. All control larvae ecdysed to the 5th instar by 84 h after feeding, and no larvae ecdysed into the 6th instar.

All larvae treated with 0·5 and 0·25 mg kg^{-1} RH-2485 died by 84 and 120 h after feeding on the test diets, respectively, and 18% larvae treated with 0·125 mg kg^{-1} remained alive until 144 h after feeding when the experiment was terminated (Fig. 4). In contrast, 10% and 80% larvae treated with 0·25 and 0·125 mg kg^{-1} tebufenozide remained alive until 144 h after feeding. No mortality was observed in the control larvae until 144 h after feeding was initiated.

2.4 Chronic effects

RH-2485 applied at 0·005 mg kg^{-1} caused significantly higher larval mortality than occurred in the control, and larval mortality increased significantly when the concentration of RH-2485 was 0·01 mg kg^{-1} ($P < 0·05$) (Fig. 5A). Fifty percent pupation was significantly delayed in larvae treated with 0·01 mg kg^{-1} RH-2485 compared with larvae treated with 0·005 mg kg^{-1} or control larvae ($P < 0·05$) (Fig. 5B). The mean weights of male and female pupae obtained from larvae exposed to 0·01 mg kg^{-1} RH-2485 were significantly lower than those from the control larvae ($P < 0·05$) (Fig. 5C). In addition, larval exposure to a sublethal concentration of RH-2485 (0·01 mg kg^{-1}) caused a significant decrease in adult emergence ($P < 0·05$) (Fig. 5D).

A similar situation was observed when newly hatched larvae were exposed to sublethal concentrations of tebufenozide. Tebufenozide applied at 0·03 and 0·06 mg kg^{-1} caused significantly higher larval mortality than occurred in the control ($P < 0·05$) (Fig. 6A). Fifty percent pupation was significantly delayed in larvae treated with 0·06 mg kg^{-1} tebufenozide compared with control larvae ($P < 0·05$) (Fig. 6B). The mean weights of male pupae obtained from control and treated larvae were not significantly different (Fig. 6C). By contrast, the mean weight of female pupae derived from control larvae was significantly greater than that of pupae from larvae treated with 0·06 mg kg^{-1} ($P < 0·05$) (Fig. 6C). Larval exposure to sublethal concentration of tebufenozide caused a significant decrease in adult emergence ($P < 0·05$) (Fig. 6D). Increasing the exposure time to tebufenozide tended to increase the larval mortality, significantly retarded larval growth ($P < 0·05$), and decreased the mean weights of male and female pupae and adult emergence ($P < 0·05$). No effect of combinations between concentrations of tebufenozide and exposure periods was detected.

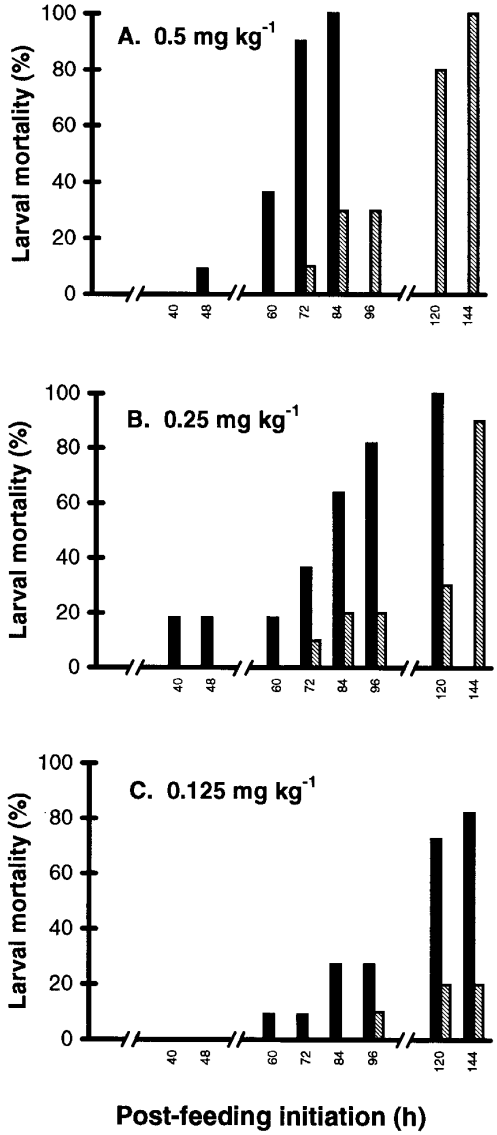

Fig. 4. Effect of (■) RH-2485 and (▨) tebufenozide administered in artificial diet on mortality of newly ecdysed 4th-instar (<1 day old) larvae of *Diatraea grandiosella* maintained at 30°C, 16 : 8 h light : dark. No mortality was observed in the control treatment until 144 h after feeding was initiated. Observation was carried out with 8 h interval in the first two days and 12 h interval in the second two days after exposure. $n = 11$ larvae per treatment for RH-2485, and $n = 10$ larvae per treatment for tebufenozide.

2.5 Stability of the ecdysone agonists in the diet

RH-2485 and tebufenozide were lethal to newly hatched larvae, even after diets containing these compounds were held for 20 days at 30°C, 16 : 8 h light : dark. All larvae died when treated with 0·125 or 0·25 mg kg^{-1} RH-2485 or 0·25 or 0·5 mg kg^{-1} tebufenozide. Most larvae died during the 1st molting cycle. A few larvae (6·7%) fed with fresh diet (from day 0) containing 0·25 mg kg^{-1} tebufenozide and 3·3% of larvae treated with diet containing 0·125 mg kg^{-1} RH-2485 held for

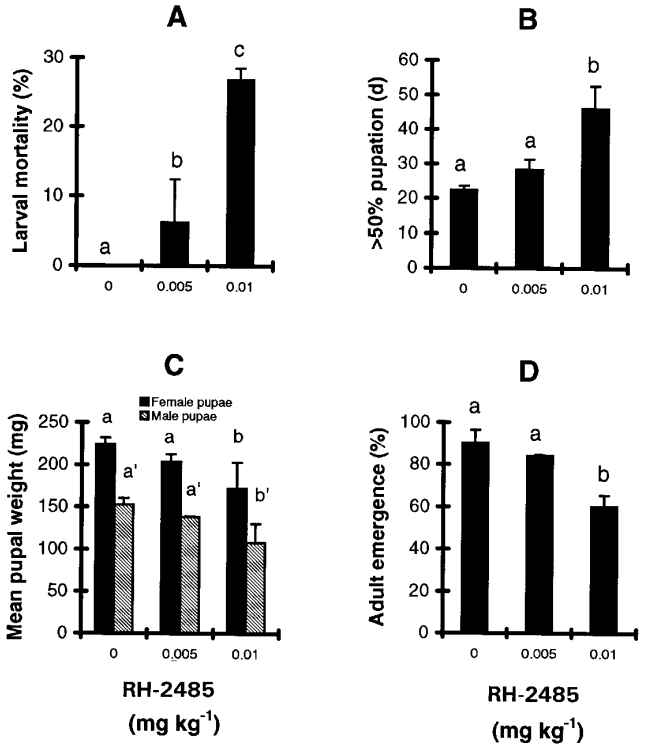

Fig. 5. Chronic effects of sublethal concentrations of RH-2485 administered in an artificial diet on growth and development of newly hatched larvae of *Diatraea grandiosella* maintained at 30°C, 16 : 8 h light : dark. Bars designated with the same letters are not significantly different, Fisher protected LSD; $P > 0{\cdot}05$. $n = 110$–111 larvae per treatment.

five days reached the 2nd instar, but they eventually died during the second molting cycle. Only one larva (3·3%) died on control diet held for 15 days.

4 DISCUSSION

RH-2485 was about four times more lethal to newly hatched larvae of *D. grandiosella* than was tebufenozide. Similarly, tebufenozide has been demonstrated to be more lethal than RH-5849 in several species of Lepidoptera.[7,8] The difference in toxicity between these ecdysone agonists may be caused by differences in their binding affinity to the receptors.[16] Using *in-vitro* cultured imaginal wing discs of the greater wax moth, *Galleria mellonella* L., tebufenozide was found to have 40 times more affinity for the receptor than had RH-5849.[16]

Significant progressive mortality was observed in larvae of *D. grandiosella* treated with RH-2485 or tebufenozide, which may be due to continuous ingestion of the compounds by the larvae, or the stability of the compounds in the larval body, or because both situations exist. Our data show that the compounds remained active even after the diet containing the compounds was held for 20 days at 30°C under long days.

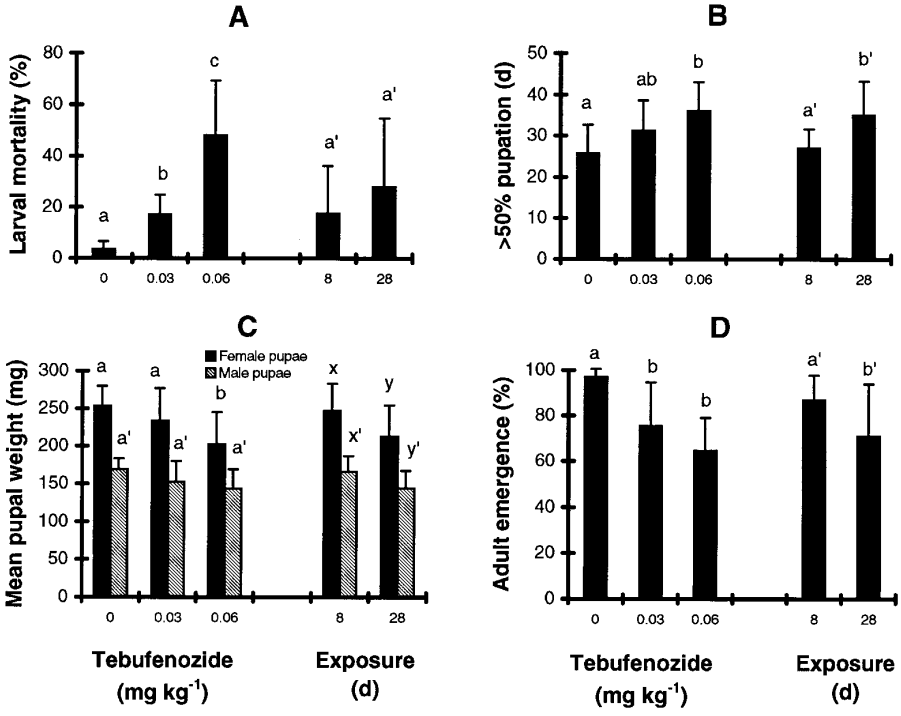

Fig. 6. Chronic effects of combinations of sublethal concentrations of tebufenozide administered in an artificial diet and exposure periods on growth and development of newly hatched larvae of *Diatraea grandiosella* larvae maintained at 30°C, 16 : 8 h light : dark. Bars indicated by the same letters are not significantly different, Fisher protected LSD; $P > 0{\cdot}05$. $n = 121$–150 larvae per treatment.

In the larval body of *S. exempta* and *S. exigua*, 8 and 17% of tebufenozide, respectively, was present as the parent compound at 4 h after uptake.[17] Therefore, continuous ingestion by larvae of *D. grandiosella* receiving sublethal concentrations of RH-2485 or tebufenozide may result in a sufficient accumulation to induce a lethal molting cycle.

Newly ecdysed 4th-instar larvae of *D. grandiosella* stopped feeding approximately 8 h after being exposed to an artificial diet containing RH-2485 or tebufenozide, indicating that larvae had prematurely entered a molting cycle. The ecdysis occurred earlier in larvae treated with RH-2485 than in those treated with tebufenozide. In addition, larvae of *D. grandiosella* treated with RH-2485 died more quickly than did those treated with tebufenozide. These results show that RH-2485 has a greater potency than does tebufenozide. These effects have implications for the use of RH-2485 as an IGR because larval feeding damage to maize would be limited.

Most larvae of *D. grandiosella* exposed as 4th instars to 0·5 mg kg^{-1} RH-2485 or tebufenozide, did not ecdyse successfully into 5th instars. Those larvae that did complete their ecdysis into the 5th instar did not gain additional weight. Similar symptoms caused by tebufenozide or RH-5849 have been reported in several species of Lepidoptera.[4,8,17–19] The failure of treated larvae to shed the old cuticle may be due to inhibition of eclosion hormone caused by residues of RH-2485 or tebufenozide in the larval body. This neurohormone is released as a result of a decline in ecdysteroid titer following the peak titer before ecdysis.[20] Feeding cessation and malformation of the mouth parts[19] may cause starvation which eventually leads to death. Some larvae treated with lower concentrations (0·25 and 0·125 mg kg^{-1}) of the compounds successfully ecdysed to the 5th instar and continued to feed on the diet. This feeding resulted in weight gain and accumulation of the compounds in the larval body which eventually induced the larvae to undergo a premature and lethal molting cycle to the 6th instar. Even though some treated larvae were alive 120 h after treatment, they were significantly smaller than control larvae.

Our results confirm and extend previous reports[9–11] showing that RH-2485 was more lethal than was tebufenozide against *S. littoralis*, *P. pyrusana*, *C. rosaceana* and *O. nubilalis*. A field trial showed that RH-2485 applied at a half-rate of tebufenozide (140 and 280 g AI ha^{-1}, respectively) against *O. nubilalis* resulted in a similar level of control.[21] Comparative field trials are necessary to determine the performance of these compounds for controlling *D. grandiosella* and their impact on the natural enemy complex. RH-2485 or tebufenozide should be applied at the time of egg hatch. Larvae that do not receive a lethal dose may suffer from sublethal chronic effects, including lower larval growth rate and decreased pupal weight and adult emergence. These effects may be significant in decreasing the population of *D. grandiosella* in the following generation. If these compounds are applied to larvae of the diapause generation, they may increase the mortality rate and decrease the number of spring adults. The selectivity and activity of these ecdysone agonists may give an alternative control strategy that is compatible with other control tactics for pest management programs in maize.

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REFERENCES

1. Chippendale, G. M., The southwestern corn borer, *Diatraea grandiosella:* case history of an invading insect. *Research Bulletin 1031*, University of Missouri, Agricultural Experiment Station, Columbia, MO, 1979.
2. Anonymous, Corn, sorghum, sugarcane. *Coop. Plant Pest Rep.*, **1** (1976) 49–50.
3. Anonymous, Estimated losses and production costs attributed to insects and related arthropods—1976. *Coop. Plant Pest Rep.*, **3** (1978) 91–117.
4. Wing, K. D., Slawecki, R. A. & Carlson, G. R., RH 5849, a nonsteroidal ecdysone agonist: effects on larval Lepidoptera. *Science* (*Washington*), **241** (1988) 470–2.
5. Heller, J. J. & Mattioda, H., Field evaluation of RH 5992 on lepidopterous pests in Europe. *Proc. Brighton Pest Cont. Conf.—Pests and Diseases*, **1** (1992) 59–65.
6. Brown, J. J., Effect of a nonsteroidal ecdysone agonist, tebufenozide, on host parasitoid interactions. *Arch. Insect Biochem. Physiol.*, **26** (1994) 235–48.
7. Smagghe, G. & Degheele, D., Action of the nonsteroidal ecdysteroid mimic RH 5849 on larval development and adult reproduction of insects of different orders. *Invert. Reprod. Develop.*, **25** (1994) 227–36.
8. Smagghe, G. & Degheele, D., Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. *Pestic. Sci.*, **42** (1994) 85–92.
9. Ishaaya, I., Yablonski, S. & Horowitz, A. R., Comparative toxicity of two ecdysteroid agonists, RH 2485 and RH 5992, on susceptible and pyrethroid-resistant strains of the Egyptian cotton leafworm, *Spodoptera littoralis. Phytoparasitica*, **23** (1995) 139–45.
10. Brunner, J. F., Doerr, M. D. & Smith, L. O., Bioassay, effects of molt accelerating compounds (MAC) on neonate leafroller larvae, 1994, *Arthropod Manag. Test*, **20** (1995) 343–4.
11. Trisyono, A. & Chippendale, G. M., Effect of the nonsteroidal ecdysone agonists, methoxyfenozide and tebufenozide, on the European corn borer (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **90** (1997) 1486–92.

12. Hsu, A. C., 1,2-diacyl-1-alkylhydrazines: a new class of insect growth regulators. In *Synthesis and Chemistry of Agrochemicals II*, ed. D. R. Baker, J. G. Fenyes & W. K. Moberg. American Chemical Society, Washington, DC, 1992, pp. 478–90.
13. Chippendale, G. M. & Cassatt, K. L., *Diatraea grandiosella*. In *Handbook of Insect Rearing*, *vol. 2*, ed. P. Singh & R. F. Moore, Elsevier Science Publishers, Amsterdam, 1985, pp. 257–64.
14. Gomez, K. A. & Gomez, A. A., *Statistical procedures for agricultural research*, *with emphasis on rice*. International Rice Research Institute, Los Banos, Philippines, 1976.
15. Eisensmith, S. & Russel, D. F., MSTAT-C: a microcomputer program for the design, management and analysis of agronomic research experiment. MSTAT development team, Michigan State University, East Lansing, MI, 1989.
16. Smagghe, G., Eelen, H., Verschelde, E., Richter, K. & Degheele, D., Differential effects of nonsteroidal ecdysteroid agonists in Coleoptera and Lepidoptera: analysis of evagination and receptor binding in imaginal discs. *Insect Biochem. Molec. Biol.*, **26** (1996) 687–95.
17. Smagghe, G. & Degheele, D., The significance of pharmacokinetics and metabolism to the biological activity of RH 5992 (tebufenozide) in *Spodoptera exempta*, *Spodoptera exigua* and *Leptinotarsa decemlineata*. *Pestic. Biochem. Physiol.*, **49** (1994) 224–34.
18. Darvas, B., Polgar, L., El-din, M. H. T., Eross, K. & Wing, K. D., Developmental disturbances in different insect orders caused by an ecdysteroid agonist, RH 5849. *J. Econ. Entomol.*, **85** (1992) 2107–12.
19. Smagghe, D. & Degheele, D., Effect of RH 5849, the first nonsteroidal ecdysteroid agonist, on larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Arch. Insect Biochem. Physiol.*, **21** (1992) 119–28.
20. Truman, J. W., Rountree, D. B., Reiss, S. E. & Schwartz, L. W., Ecdysteroids regulate the release and action of eclosion hormone in the tobacco hornworm, *Manduca sexta* (L.). *J. Insect Physiol.*, **29** (1993) 895–900.
21. Seymour, R. C., Campbell, J. B. & Wright, R. J., Control of European corn borer larvae on reproductive stage field corn, 1994. *Arthropod Manag. Test*, **21** (1996) 228.